Production of Mustard Protein Isolates from Oriental Mustard Seed (*Brassica juncea* L.)

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ABSTRACT: A membrane-based process to produce protein isolates from seeds of oriental mustard (*Brassica juncea*) was developed by modifying a method originally developed for rapeseed. The optimized process consisted of extraction at pH 11, ultrafiltration with concentration factor 4, diafiltration with diavolume 3, and precipitation at pH 5. The process, based on defatted oriental mustard seed containing 45–50% protein, recovered 81% of the protein in useful products: 47.3% in precipitated protein isolate (PPI), 3.8% in soluble protein isolate (SPI), and 13% in meal residue. Mass yields were 21.9% in PPI, 2.8% in SPI, and 38.4% in meal residue. The losses in the system included ~10% loss of nonprotein nitrogen, and <9% into permeate and transfer losses. The PPI compared favorably with soy protein isolate in typical meat products in terms of color, texture, and flavor. The work confirms that oriental mustard is a potentially useful source of edible protein.

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KEY WORDS: Diafiltration, extraction, membrane processes, mustard proteins, oriental mustard, protein isolates, ultrafiltration.

The relatively high protein content (18–24%) of rapeseed and mustard seed varieties makes them attractive potential sources of food-grade vegetable protein. The balance of amino acids found within the seed of these crops compares favorably with that required for human nutrition. Several methods have been examined for the production of protein isolates (>90% protein) and concentrates (>65% protein) from these crops. Procedures that produce isolates generally involve oil extraction, solubilization of protein, purification, precipitation, and drying. To be useful as a food ingredient, an isolate must have good food functionality, a well-balanced amino acid composition, and acceptable organoleptic properties.

Unfortunately, seeds from the Brassicaceae contain several antinutritional and flavor components that tend to bind to the protein and are carried to the isolate. These components include glucosinolates and their toxic breakdown products, phenolics and phytates, which hinder bioavailability of amino acids and minerals (1–3). These components are also largely responsible for the dark color and strong, astringent flavor of the products; therefore, they must be substantially removed.

Tzeng *et al.* (4) used canola seed (Altex and Westar cultivars) to successfully develop a process that produced three products: an isoelectric protein isolate, a soluble protein isolate, and a meal residue. The protein isolates were high in protein, free of glucosinolates, and low in phytates. Xu and Diosady (5) adapted this process to Chinese rapeseed and further modified it to reduce the phenolic content of the protein products (6). This approach has since been adapted and modified for yellow mustard (7,8). The procedure makes use of membrane processing in a series of stages that first liberates the phenolics from the protein, then concentrates the protein in the extract solution and reduces the levels of unbound antinutritional components through ultrafiltration and diafiltration. Pilot scale tests on this approach using yellow mustard as the raw material were successfully completed (7).

While both are members of the same family, oriental mustard (*Brassica juncea*) is notably different from yellow mustard (*Sinapis alba*). The two seeds contain different glucosinolates, and the oriental mustard is darker in color, owing in part to its high phenolic content. Unlike yellow mustard, oriental mustard does not have a mucilaginous coating around the seed. The oil content of the oriental seed is also significantly higher.

To develop a viable protein recovery process, this work involved determination of the influence of pH on protein extractability and precipitation. From these results, optimal pH values were chosen for the extraction and precipitation stages, and the appropriate concentration factor for ultrafiltration and the diavolume (DV) for diafiltration were defined. The functionality of the isolate in a typical processed meal application was compared with the performance of a commercial soy protein isolate in terms of texture, flavor, and color.

MATERIALS AND METHODS

Whole oriental mustard seed, lot #202, was obtained from G.S. Dunn & Co. Limited (Hamilton, Ontario, Canada). Prior to use, the seed was ground and defatted with hexane, using a Soxhlet apparatus, for 24 h, and then dried overnight in a fume hood. The composition is presented in Table 1.

Sodium hydroxide, 50% aqueous solution, and phosphoric acid, ~38%, were diluted as required. Analytical-grade sodium chloride, ascorbic acid, and other reagents required for analytical work were obtained from VWR International (Mississauga, Canada), BDH (Toronto, Canada), and Fisher Scientific (Fairlawn, NJ).

A hotplate stirrer was used to mix material produced in small batches and to heat all material as required in the process.

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TABLE 1
Composition of Ground Oriental Mustard Seed

Material	Crude protein[a] % (N × 6.25)	Moisture %	Oil %	Isothiocyanates[b] μmol/g	Phytates[c] %	Phenolics[b] mg/100 g (as sinapic acid)
Ground oriental mustard seed	29.0 ± 0.2	5.2 ± 0.2[d]	37 ± 4[e]	N/A	N/A	N/A
Defatted ground oriental mustard seed	45.0 ± 0.2	8.3 ± 0.2[f]	N/A	169 ± 5	3.3 ± 0.3	1690 ± 80

[a]Mean ± SD of three sets of triplicates.
[b]Mean ± SD of six replicates.
[c]Mean ± SD of four replicates.
[d]Mean ± SD of six sets of triplicates.
[e]Mean ± SD of 75 individual measurements.
[f]Mean ± SD of triplicates. N/A, not analyzed.

Large batches of material were mixed with a type RZR50 stirrer (Caframo, Wiarton, Canada).

The protein extractability was determined by contacting 10-g portions of seed with aqueous NaOH at a solvent-to-seed ratio of 18 for 30 min with continuous stirring at a preselected pH level, ranging from 7 to 13. The pH was adjusted using 25% NaOH. The extract and solids were separated by centrifugation at 9000 × *g*. The liquid was decanted and vacuum-filtered through Whatman 541 paper to a receiving flask. The solids were then washed twice with water (water-to-seed ratio of 6), each time decanting through the filter paper into the same receiving flask. The extractability was measured as the mass ratio of protein recovered in the collected extract solution compared with that in the 10 g of starting material.

The effect of pH on precipitated protein recovery was determined by adjusting the pH of 200 g of extract solution prepared at pH 11 using 3 M phosphoric acid and maintaining the selected pH for 30 min. The precipitate was separated from solution by centrifugation and washed with 10 g of water. The protein content of the washed precipitate was measured, as was the protein content of the combined liquid.

Batches of pH 11 extract were prepared for the investigation of membrane processing stages. The extract was treated with 0.05 M NaCl, heated at 55°C for 30 min, and cooled to 40°C. Ultrafiltration of the heat-treated pH 11 extract was conducted by using a Millipore Pellicon™ system, which consists of a cartridge holder, pressure gauge and valve, peristaltic pump, and digital control drive. A 5 kDa regenerated cellulose membrane (PLCCC; Millipore, Cambridge, Ontario, Canada) was used in this process, and the concentration factor (C_F) was varied. The C_F for a given molecule type is defined after Cheryan (9) as

$$C_F R = C_F / C_O \quad [1]$$

where C_O is the initial concentration in the feed, C_F is the final concentration in the retentate, and R is the rejection coefficient, which relates to how well the component is able to pass through the membrane. Also,

$$R = 1 - C_p / C_r \quad [2]$$

where C_p is the concentration in the permeate and C_r is the concentration in the retentate.

Batches of extract were processed to attain a C_F of 2, 3, 4, or 5. The retentate was then precipitated at pH 5. The wet isolates were analyzed for protein and glucosinolate contents. Consistency of the retentate and color of isolates were also observed.

Diafiltration was then performed at DV of 3 and 6, with 0.05 M aqueous NaCl, using the same equipment as for ultrafiltration. The treated solution was acidified, and the precipitated solids were recovered and analyzed for protein. Their color was also observed.

The process was repeated several times under the optimized conditions, and the resulting products were combined. The precipitated protein isolate (PPI) and meal residue were tested as binders in the production of wieners and bologna following typical production procedures.

Standard analytical techniques were used. Crude protein was determined by the Kjeldahl method and reported as N × 6.25, according to method 46-12 (10) of the American Association of Cereal Chemists (AACC) (10). This factor was adopted, as it is the standard used by the food and feed industry. The concentration of glucosinolate hydrolysis products was determined by the method of Wetter and Youngs (11). Because isocyanates are the primary breakdown products of benzyl glucosinolates, and as the flavor of the product depends on the isothiocyanates—determination of glucosinolate gives the best reflection of product quality. The phenolic content was determined according to Xu and Diosady (6). The phytate content was determined according to Naczk *et al*. (1). Oil content was determined gravimetrically, using Soxhlet extraction with petroleum ether for a total of 24 h. Moisture was determined gravimetrically by AACC method 44-15A (10).

RESULTS AND DISCUSSION

Protein extractability. Crude protein, which includes nonprotein nitrogen and overestimates the actual protein content of oilseeds, was analyzed, and all references to protein quantities in this work refer to crude protein, as defined by the analytical method.

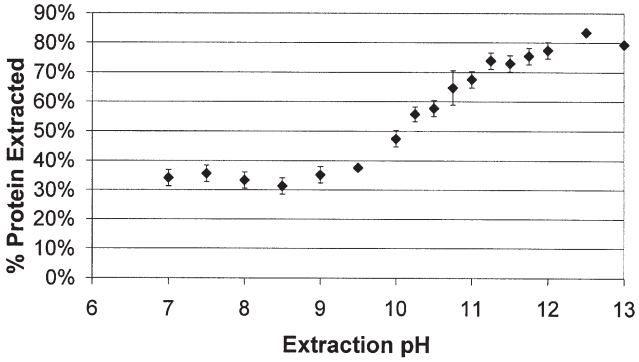

FIG. 1. Protein extractability from defatted ground oriental mustard seed by aqueous sodium hydroxide solutions as a function of pH.

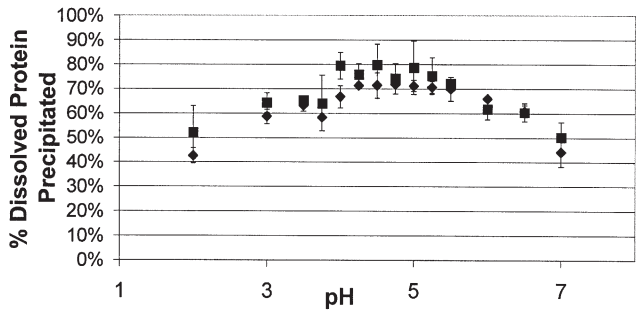

FIG. 2. Precipitation of ground mustard seed proteins using aqueous solutions of phosphoric acid as a function of pH. (■) Calculated from protein analysis of precipitate; (◆) calculated as mass balance from protein analysis of combined effluent.

Protein extractability increased with increasing pH above pH 9. The maximum extractability, 83%, was reached at pH 12.5 (Fig. 1). Operating above pH 11 was impractical, as the ultrafiltration membrane used was unstable above pH 11, and operating above pH 11 required significantly higher amounts of NaOH, which would have to be neutralized later by acid addition. Exposing the protein to high pH values also increased the risk of lysinoalanine formation, which could render the isolate toxic. We therefore selected pH 11 for all further extraction experiments.

Dissolved protein precipitation. The amount of precipitate formed is highest between pH 4 and pH 5.5 (Fig. 2). Since lower pH requires more acid and tends to render the solution more difficult to stir as precipitation occurs, pH 5 was selected for further studies.

All precipitates produced after ultrafiltration contained >90% crude protein (N × 6.25) and no detectable levels of glucosinolates/isothiocyanates. The crude protein content of the isolates produced with a C_F of 4 and 5 were highest, at 95%. It was visually observed that as C_F increased from 2 to 5, the color of the isolate became increasingly pale. The processing of the solution became more difficult with increasing C_F, especially between C_F 4 and 5. Therefore, a C_F of 4 was selected for the final process.

Further purification was accomplished with diafiltration using a DV of 3. Increasing the DV to 6 made no noticeable improvement in either the color of the isolate or the protein content. The isolates that were made without diafiltration were slightly darker than those that were diafiltered.

Selected process. The final isolation procedure for the oriental mustard, as shown in Figure 3, consisted of aqueous alkaline extraction at pH 11 and a water-to-seed ratio of 18 for 30 min, followed by two washing stages using a water-to-seed ratio of 6 each time. The solids were dried to produce a meal residue. Prior to drying, the pH may be adjusted to make the product more suitable as a food ingredient. The extract was treated with 0.05 M NaCl and heated at 55°C for 30 min.

The heat-treated extract is concentrated by ultrafiltration at C_F = 4 and purified by diafiltration with DV = 3. Aqueous 0.05 M NaCl adjusted to pH 10.5 with NaOH was used for the diafiltration step to prevent protein precipitation during processing. The retentate from diafiltration was acidified to pH 5 to precipitate the majority of the proteins. The precipitate was then washed with 30 g water per 100 g of starting material, and then dried to form the PPI. The mother liquor

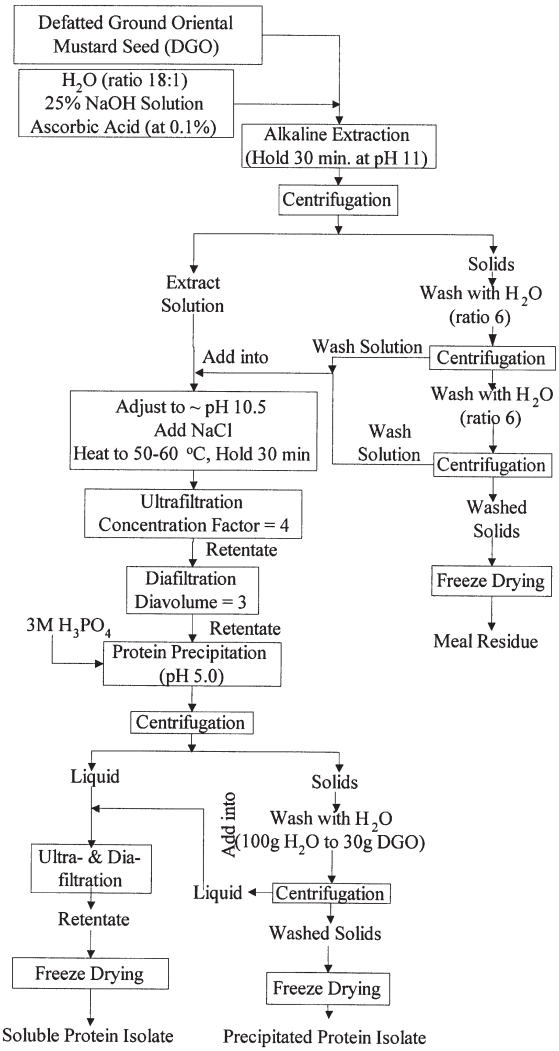

FIG. 3. Process flow schematic, indicating the steps for producing the final protein products from defatted ground oriental mustard seed.

TABLE 2
Composition of the Starting Defatted Oriental Mustard Seed and the Final Products

Products	Crude protein[a] % (N × 6.25)	Moisture[a] %	Isothiocyanates[b] μmol/g	Phytates[c] %	Phenolics[b] mg/100 g (sinapic acid)
Defatted mustard seed	45.0 ± 0.2[d]	8.3 ± 0.2[d]	169 ± 5	3.3 ± 0.3	1690 ± 80
Meal residue	36.0 ± 0.1	7.4 ± 0.1	<5	2.9 ± 0.3	
Precipitated protein isolate	96.0 ± 0.1	4.3 ± 0.1	<5	<0.5	168 ± 90
Soluble protein isolate	72.0 ± 1.0	N/A	N/A	N/A	N/A

[a]Mean ± SD of triplicates.
[b]Mean ± SD of six replicates.
[c]Mean ± SD of four replicates.
[d]Mean ± SD of three sets of triplicates. N/A, not analyzed owing to insufficient sample size.

was further concentrated by ultrafiltration with a C_F of 3 and diafiltered at a DV of 2, using pure water, before dewatering to form soluble protein isolate (SPI). Table 2 summarizes the product characteristics obtained using this procedure. Both isolates and meal residue were pale in color. The SPI was slightly salty and on average had a lower than desirable crude protein content of 72%. However, this could be improved by further diafiltration. The PPI contained 96% crude protein, no detectable glucosinolates or phytates, and 168 ± 90 mg sinapic acid/100 g sample (mean ± SD). The PPI had little flavor, although it had a somewhat astringent taste.

The meal residue contained 36% protein and thus had potential to be used in applications where deheated mustard is currently used.

Functionality testing. Three standard meat emulsions were prepared using the mustard PPI, meal residue, or soy protein isolate as added binder. Some of each emulsion was stuffed into fibrous casings and then smoked; the rest were stuffed into waterproof casings and cooked. Comparison of the meat products was accomplished by the ranking of each property on a separate unstructured scale by a small expert panel and a larger nonexpert panel (12). The wieners and bologna thus produced using either PPI from oriental mustard or meal residue as the binding agent compared favorably with those produced using SPI. In terms of color, the oriental PPI was darker than the others. However, in terms of texture and flavor there was statistically no difference between any of the products.

As demonstrated by the foregoing, a protein isolation procedure was successfully developed using defatted ground oriental mustard seed as the starting material. The total crude protein recovery in the three product streams was 81%. The distribution of protein among the three products is shown in Figure 4. The PPI was of high quality and had excellent crude protein content (96%) and low concentrations of antinutritional components. Nearly half of the protein in the seed (47.3%) was recovered as PPI. The taste of this product was bland, and the color was only slightly darker than yellow mustard isolates. The PPI was also comparable with SPI as a meat binder in typical smoked and cooked products.

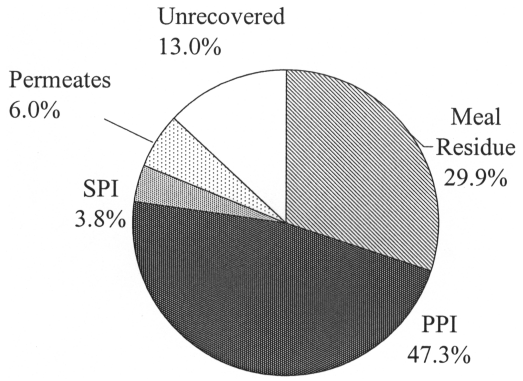

FIG. 4. The distribution of crude protein among the three products and other process streams. Values are based on the average of triplicate runs. SPI, soluble protein isolate; PPI, precipitated protein isolate.

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REFERENCES

1. Naczk, M., L.L. Diosady and L.J. Rubin, The Phytate and Complex Phenol Content of Meals Produced by Alkanol–Ammonia/Hexane Extraction of Canola, *Lebensm. Wissen. Technol. 19*:13–16 (1986).
2. Dijkstra, D.S., A.R. Loinnemann, and T.A.J.S. van Boekel, Towards Sustainable Production of Protein-Rich Foods: Appraisal of Eight Crops for Western Europe. Part II: Analysis of the Technological Aspects of the Production Chain, *Crit. Rev. Food Sci. Nutr. 4*:481–506 (2003).
3. Naczk, M., P.K.J.P.D. Wanasundara, and F. Shahidi, Facile Spectrophotometric Quantification Method of Sinapic Acid in Hexane-Extracted and Methanol-Ammonia-Water-Treated Mustard and Rapeseed Meals, *J. Agric. Food Chem. 40*:444–448 (1992).
4. Tzeng, Y.M., L.L. Diosady, and L.J. Rubin, Production of Canola Protein Materials by Alkaline Extraction, Precipitation,

and Membrane Processing, *J. Food Sci. 55*:1147–1156 (1990).
5. Xu, L., and L.L. Diosady, The Production of Chinese Rapeseed Protein Isolates by Membrane Processing, *J. Am. Oil Chem. Soc. 71*:935–939 (1994).
6. Xu, L., and L.L. Diosady, Interactions Between Canola Proteins and Phenolic Compounds in Aqueous Media, *Food Res. Int. 33*:725–731 (2000).
7. Diosady, L.L., L. Xu, and B.-K. Chen, Production of High-Quality Protein Isolates from Defatted Meals of *Brassica* Seed, U.S. Patent 6,905,713 (2005).
8. Xu, L., F. Lui, H. Luo, and L.L. Diosady, Production of Protein Isolates from Yellow Mustard Meals by Membrane Processes, *Food Res. Int. 36*:849–856 (2003).
9. Cheryan, M., *Ultrafiltration and Microfiltration Handbook*, Technomic, Lancaster, PA, 1998.
10. American Association of Cereal Chemists, *Approved Methods of the American Association of Cereal Chemists*, St. Paul, MN, 1976.
11. Wetter, L.R., and C.G. Youngs, A Thiourea-UV Assay for Total Glucosinolate Content in Rapeseed Meals, *J. Am. Oil Chem. Soc. 53*:162–164 (1976).
12. Larmond, E., L.M. Poste, D. Mackie, and G. Butler, Laboratory Methods for Sensory Analysis of Food, Research Branch Agriculture Canada, Publication 1864/E (1991).

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